CHAPTER IV

RESULTS AND DISCUSSION

4.1 Oil and water sorption by chitosan

Three forms of chitosan were examined for the capacity of water and oil sorption in pH 7.0 buffer solutions. To determine water sorption capacity of each form, dry chitosan was added to the buffer solution and then shaken for 1 hour. The water sorption capacity of chitosan was carried out by weighing the damp sorbents after finish shaking and then water sorption capacity was calculated as a ratio of water adsorbed to dry sorbent weight. The oil sorption capacity of chitosan was performed by adding a small amount of lubricating oil to a buffer solution hourly and shaking immediately. The cycle was repeated until oil film was formed on the free water surface at the end of a shake period and then oil sorption capacity was calculated as the ratio of oil adsorbed to dry adsorbent weight. The maximum water and oil sorption of different chitosan forms are shown in table 4.1. Results indicated that these three forms of chitosan have the potential to sorp oil from water phase. Among three forms of chitosan, powder chitosan exhibited the highest water and oil sorption capacity, followed by flake squid pen chitosan and flake shrimp shell chitosan. Thus, powder chitosan was selected to investigate the optimum oil sorption condition in further study.

The high water and oil sorption of powder chitosan could be described by the high surface area of powder chitosan. Ahmad *et al.* (2005) found that the BET surface area of powder and flake chitosan was 20-30 and 5-10 m^2/g respectively. Furthermore, the high oil sorption capacity of powder chitosan was also corresponded with the study of Ahmad *et al.* (2005), in which the flake chitosan has lesser oil sorption capacity than powder chitosan when apply to palm oil mill effluent. They suggested that chitosan may bind residue oil with its amino sites near the outer surface. Eventually when time goes by, the sorbed residue oil starts to clog the pores near the outer surface so residue oil can no longer diffuse to the active sites deep within the interior surface (Ahmad *et al.*, 2005).

Table 4.1 Water and oil sorption capacities of chitosan

Sorbent	Water absorbency (g water/g chitosan)	Oil absorbency (g oil/g chitosan)
Powder chitosan	10.13 ± 0.48	0.48 ± 0.04
Flake shrimp shell chitosan	1.96 ± 0.62	0.19 ± 0.02
Flake squid pen chitosan	2.17 ± 0.70	0.24 ± 0.03

4.2 Optimum condition for oil-in-water emulsion treatment by chitosan

4.2.1 Effect of chitosan dosage

The amount of powder chitosan was varied to find the lowest amount that provided maximum oil removal efficiency. Tests were conducted using 0, 0.1, 0.5, 1.0, 3.0, and 5.0 g/L of powder chitosan. The effect of powder chitosan dosage on the removal of oil-in-water emulsion was studied with 200 mg/L oil-in-water emulsion and 60 min mixing time. The percent of oil removal was intensely increased from 66% to 80% when the concentration of powder chitosan was increased from 0.1 to 1.0 g/L (Figure 4.1). However, when the concentration of powder chitosan was increased from 1.0 to 5.0 g/L, the percentage of oil removal was relatively stable.

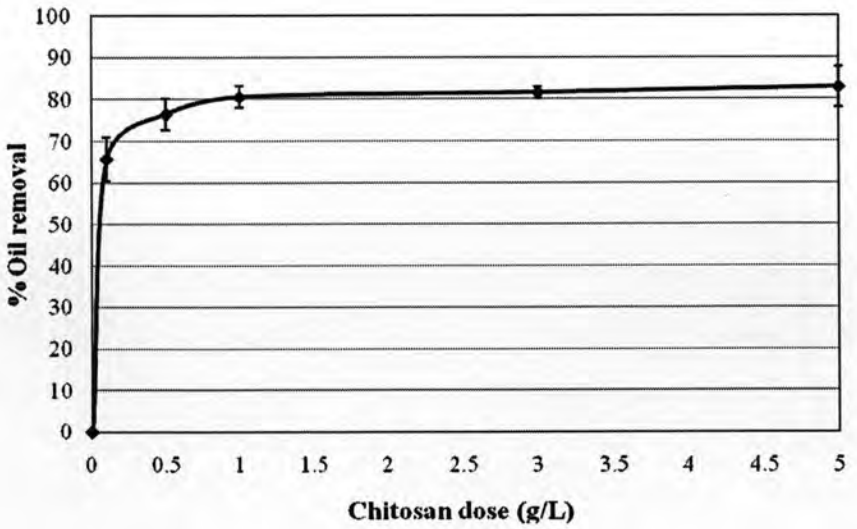


Figure 4.1 Percentage of oil removal after treatment vs. dosage of chitosan

The initial chitosan dosages also affected the removal of turbidity, which was used to represent the amount of oil-in-water emulsion. When the amount of powder chitosan was increase from 0.1 to 1.0 g/L, percent turbidity removal was increased from 45% to 78% (Figure 4.2). This indicated that powder chitosan was very efficient in removing oil emulsion. It was also seen that a powder chitosan dosage of 1.0 g/L produced maximum clarification, with no noticeable advantage at higher dosage. Therefore, the optimum concentration of chitosan is 1.0 g/L because it was the lowest concentration of powder chitosan that provided oil and turbidity removal efficiency around 80%.

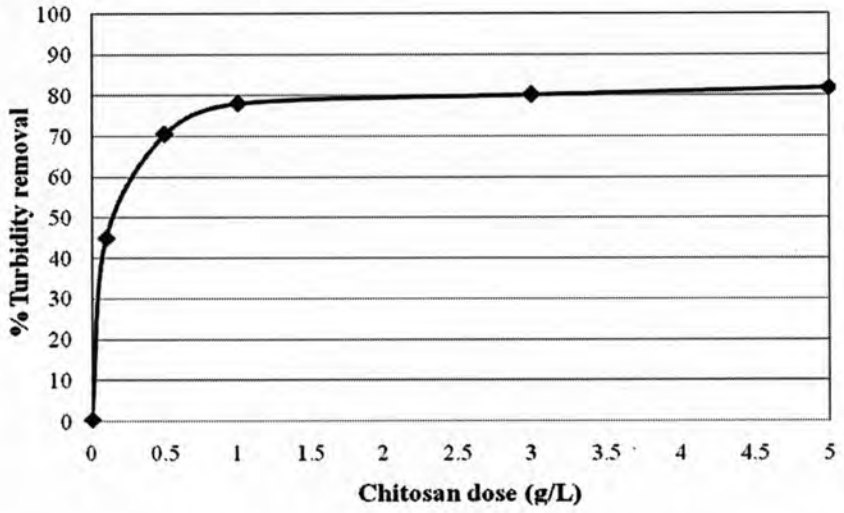


Figure 4.2 Percentage of turbidity removal after treatment vs. dosage of chitosan

The emulsion removal ability of chitosan was proablaby due to the interaction between charge of chitosan and oil-in-water emulsion. Chitosan chain structure has positively charged amine (NH_2) functional groups which are very attracted to anionic ions (Osman and Arof, 2003). Therefore, it could easily bind and bridge with negatively charged materials into a floc. The overall charge of chitosan is positive; whereas, the majority charges of oil-in-water emulsion is negative (Panpanit *et al.,* 2001). Furthermore, oil droplets in an oil-in-water emulsion are likely to have a negative charge, as described by the Helmholtz theory of the electrical double layer, which states that if the negative charges are aligned or closely bound to the interlayer, charges of the opposite type will line up parallel to them, forming an electrical double layer that causes oil droplets to repel each other (Althers, 1998). Thus, attractions

between the charges enhance the agglomeration process and this mechanism is called as charge neutralization (Ahmad *et al.,* 2005). Consequently, chitosan not only acts as sorbent but also as a coagulant.

When more than 1 g/L powder chitosan was added, the extent of oil and turbidity removal was still constant (Figure 4.2). This might be described by the mechanism of bridging of polymers. Normally, chitosan (branched polynuclear polymers) can adsorb with the active sites on the surface of colloids (oil-in-water emulsion) via charge neutralization process, and destabilize the emulsion to form large settable particles (Kumar, 2000). However, if chitosan was overdosed, the active sites on colloids were fully occupied and wrapped up by chitosan, so the colloids re-stabilization occurred, which is not favor the reaction of coagulation and decrease the removal rate (Chi and Cheng, 2006).

4.2.2 Effect of mixing time

Optimum mixing time was defined as the lowest time that chitosan needed to sorp the highest amount of oil. The effect of mixing time for oil-in-water emulsion removal by chitosan was performed with 200 mg/L oil-in-water emulsion and 1 g/L powder chitosan which is the optimum chitosan dosage from the previous experiment. Tests were conducted using 0, 10, 20, 40, 60, and 80 min of mixing time. It was found that chitosan required at least 60 min to sorb most of oil from the aqueous phase (Figure 4.3).

The effect of various mixing time on the removal of turbidity was also determined. It is also observed that 60 min mixing time produced a complete clarifIcation of aqueous phase (Figure 4.4). In addition, it was noticed that as the time was prolong from 0 to 60 min, the removal of oil and turbidity were increasing. The increasing of mixing time contributed to the higher sorption time for chitosan to remove oil-in-water emulsion.

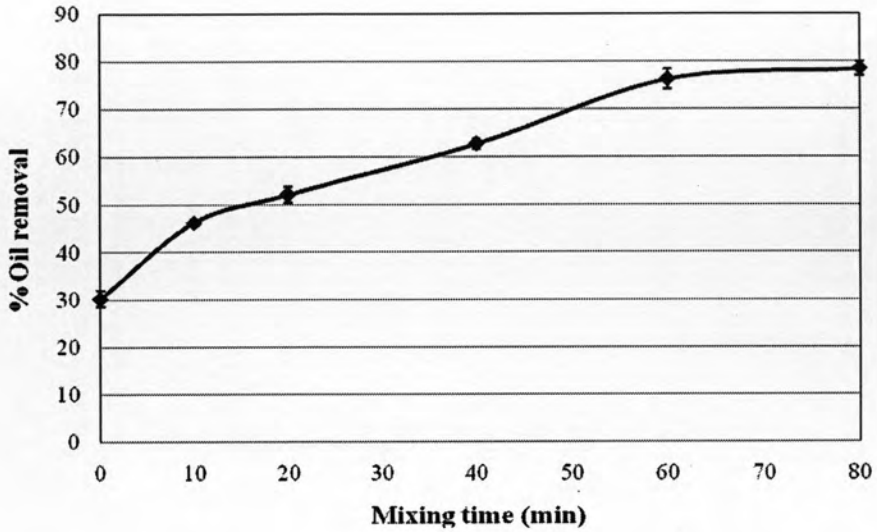


Figure 4.3 Percentage of oil removal after treatment vs. mixing time

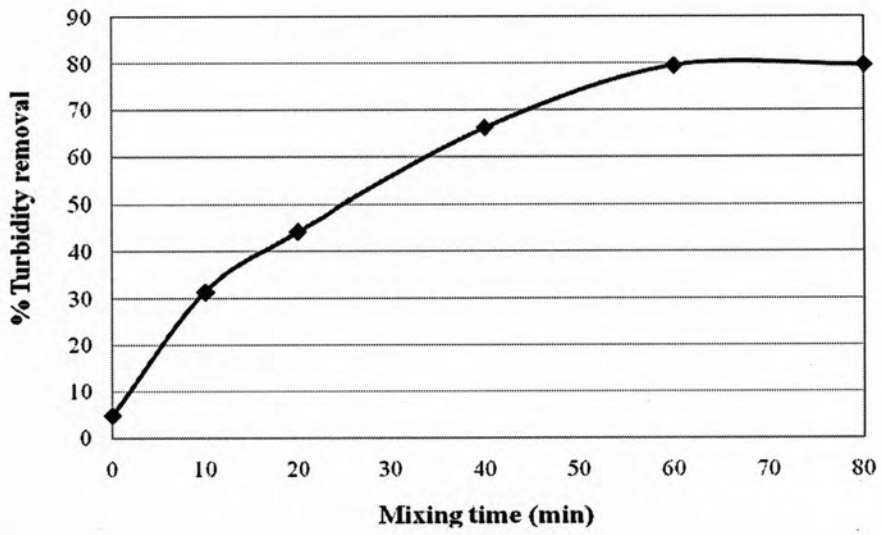
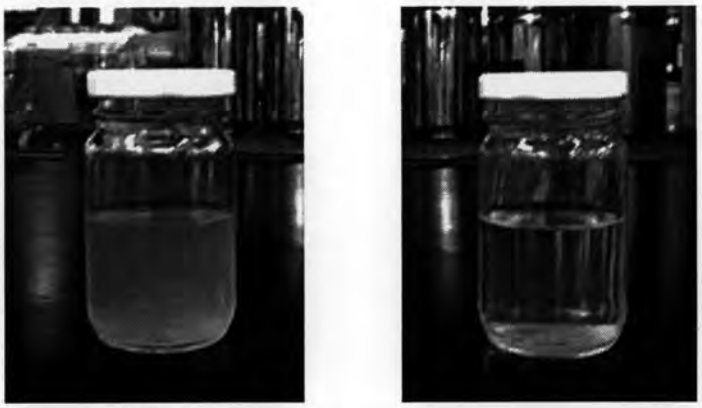


Figure 4.4 Percentage of turbidity removal after treatment vs. mixing time

From all of these results, it can be concluded that 1.0 g/L powder chitosan and 60 min mixing time were the optimum condition for oil-in-water emulsion treatment by powder chitosan. Photographs demonstrating the treatment of 200 mg/L oil-in-water emulsion by powder chitosan with the optimum condition were in Figure 4.5.



(a) (b)

Figure 4.5 The oil-in-water emulsion before (a) and after (b) treated by powder chitosan with the optimum condition (1.0 g/L powder chitosan and 60 min mixing time).

4.3 Lubricating oil degradation by isolated bacteria

Lubricating oil-degrading bacteria used in this study were isolated from soil samples collected from Chanthaburi province. Five lubricating oil-degrading bacteria, which had different colony morphology, were selected to determine the lubricating oil degradation efficiency. Tests were performed by incubated 25,000 mg/L lubricating oil with bacteria cell suspensions for 20 days. Control was the CFMM media containing lubricating oil without bacteria. The composition of lubricating oil was analyzed into saturates and aromatics by TLC-FID. There was found that bacteria Ch2 and Ch4 achieved the highest lubricating oil removal efficiency compared with the control at day 20 (Figure 4.5).

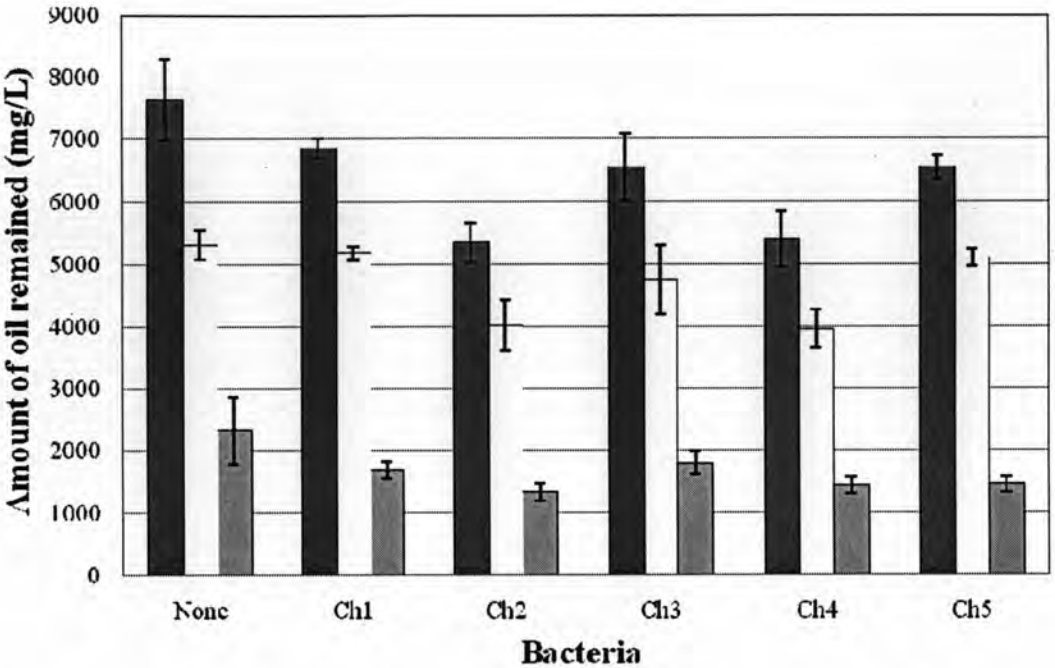


Figure 4.6 Amount of total oil (■), saturate (□), and aromatic (■) remained in CFMM after degrading by five bacteria isolated from five soil samples for 20 days.

Percent degradation of saturate and aromatic fractions in lubricating oil compared with control at day 20 was shown in table 4.2. All isolated bacteria could degrade aromatics fraction more efficiently than saturates fraction, for example the bacteria Ch2 degraded aromatic and saturate fractions at 33 and 26%, respectively. Although, the aromatics are generally more resistant to biodegradation, some low-molecular-weight aromatics such as naphthalene may be oxidized before many saturates (Venosa, 2003). Thus, it has the possibility that bacteria can degrade aromatics more than saturates. In 2002, Jirasripongpun studied the degradation of lubricating oil by seven bacteria isolated from lubricating oil contaminated soil and analyzed the composition of lubricating oil after degradation experiment into component groups of saturates and aromatics by TLC-FID method. There were two bacteria stains had the potential to degraded aromatic more than saturate fraction; whereas, other three bacteria strains had the potential to degrade saturate more than aromatic fraction. The best aromatic degrader can degrade aromatic and saturate fractions at 30 and 26% of the initial 2,000 ppm used lubricant oil, respectively. The rest of isolates could degrade saturate and aromatic fractions equally.

Table 4.2 Percent degradation of total oil, saturate and aromatic fractions comparing to control (CFMM containing lubricating oil without bacteria) at day 20th

Isolates	% Removal		
	Total oil	Saturates	Aromatics
Ch1	8.16 ± 2.04	5.72 ± 2.07	22.24 ± 5.56
Ch2	23.52 ± 4.09	26.83 ± 7.40	33.65 ± 5.72
Ch3	12.51 ± 7.13	13.57 ± 10.09	19.08 ± 8.13
Ch4	23.50 ± 5.82	27.92 ± 5.58	30.29 ± 6.01
Ch5	11.35 ± 2.61	7.19 ± 2.25	29.62 ± 5.50

Bacteria Ch2 and Ch4 were selected and later examined with oil-in-water emulsion to determine the effects of emulsifier on their oil-degrading capabilities. The experiment was performed by inoculated bacteria cell suspensions with 200 mg/L oil-in-water emulsion. Each sample was collected and analyzed for the amount of residual oil in water at 0, 1, 4, 12, and 24 h to determine the degradation efficiency of each bacterium. Number of oil-degrading bacteria survived during the experiment was also determined by total plate count to determine the relationship between oil removal and bacterial growth. Biodegradation of oil-in-water emulsion by bacteria Ch2 showed the increasing of percent oil removal until the end of the study (Figure 4.7). However, biodegradation of oil-in-water emulsion by bacteria Ch4 showed the increasing of percent oil removal in the first period and after 4 hours incubated time, percent oil removal was decreased continuously. There was found that, bacteria Ch2 degraded emulsified oil up to 80% of the initial emulsion concentration; whereas, bacteria Ch4 could degrade only 65% of the initial emulsion concentration. These results show that bacteria Ch2 had the highest oil emulsion removal efficiency. Therefore, bacteria Ch2 was selected for further study. Bacteria Ch2 were preliminary characterize by Gram's staining. The result showed that bacteria Ch2 were the mixed culture of Gram-negative rod and coccus bacteria.

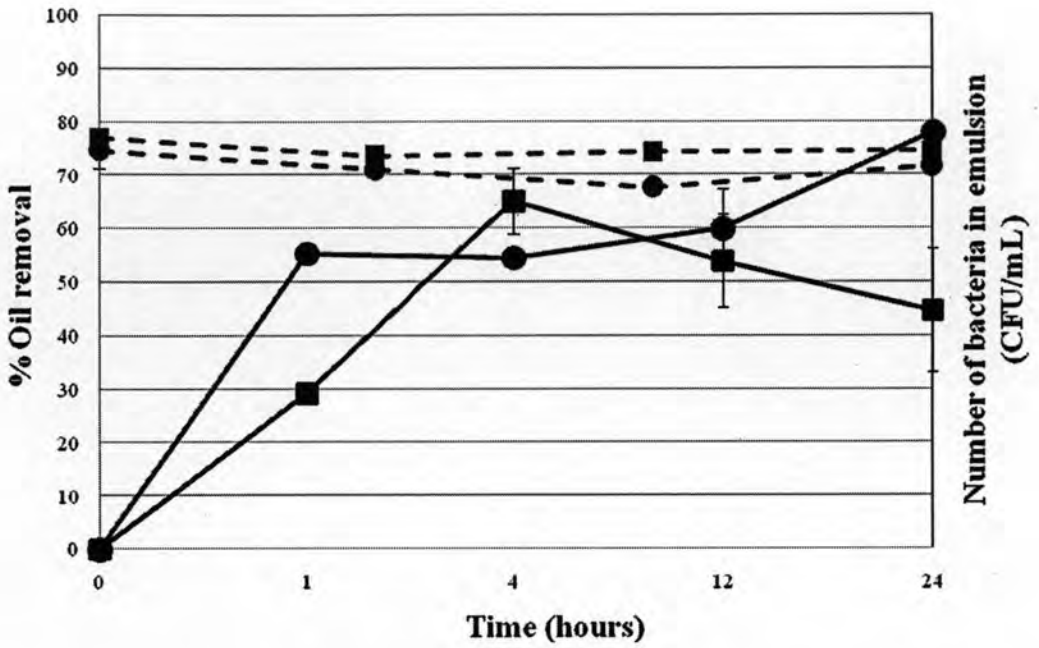


Figure 4.7 Percent oil removal by bacteria Ch2 (—●—) and Ch4 (—■—) and the number of bacteria Ch2 (- -●- -) and Ch4 (- -■- -) survival during the oil-in-water emulsion degradation experiment

The amounts of bacteria Ch2 and Ch4 strains were constant throughout the study (Figure 4.7). The oil-in-water emulsion contained only oil, emulsion, and waters thus the growth of bacteria without any nutrients (N, P) addition was slow and resulting in the constant amount of bacteria survival during the experiment. Moreover, these results indicated that the present of emulsifier did not affect the bacterial survival as well as their oil-degrading activity. However, this oil-in-water emulsion degradation study period is quite short (24 h) thus the effects of emulsifier on bacteria might be reduced. Wong *et al.* (2004) reported that *Pseudomonas aeruginosa* when combined with Tween 80 effectively enhanced the solubility and degradation of phenanthrene and they also reported that Tween 80 is biodegradable. Therefore, Tween 80 used in the preparation of synthetic oil-in-water emulsion might be degraded by bacteria Ch2 and Ch4. Moreover, the present of surfactant or emulsifier can enhance oil biodegradation, as Churchill *et al.* (1995) reported that increasing the apparent aqueous solubility of hydrocarbons by non-ionic surfactants can lead to the enhanced biodegradation rates by two *Pseudomonas saccharophila* strains.

4.4 Oil-in-water emulsion treatment by bacteria immobilized on chitosan

4.4.1 Comparison of oil-in-water removal efficiency by bacteria immobilized on three forms of chitosan

Three forms of chitosan used for the immobilization of Ch2 bacteria were powder chitosan, flake shrimp shell chitosan, and flake squid pen chitosan. The immobilize bacteria Ch2 was later tested with oil-in-water emulsion in order to know which chitosan form was the suitable media for immobilization and provided the highest oil-in-water emulsion removal efficiency. The powder and flake chitosan have a large different in particle size that may result to the different ability of chitosan-immobilized cells. Squid pen chitosan is synthesized from β-chitin (amine group aligned with the OH and CH_2OH groups); whereas, shrimp shell chitosan is synthesized from alpha-chitin (anti-parallel chain alignment) (Shepherd and Falshaw, 1997). Thus, flake squid pen and flake shrimp shell may lead to the different ability of chitosan-immobilized cells.

The immobilization process of bacteria Ch2 on chitosan was performed by cultured bacteria Ch2 with chitosan in the presence of lubricating oil as the sole source of carbon and energy. This condition favored the growth of bacterial population on the chitosan surfaces and inside it pores (Gentili *et al.*, 2006). Then, chitosan-immobilized cells was later separated and dried before used to treat oil-in-water emulsion. In this experiment, 1.0 g/L chitosan-immobilized cells were added to 200 mg/L oil-in-water emulsion at the beginning. To determine the effectiveness of chitosan-immobilized cells for a long term application, 200 mg/L lubricating oil was further added daily to the oil-in-water emulsion until finish the study. Control treatment was the oil-in-water emulsion that did not have the immobilized cells. From the experiment, free oil was observed on the water surface after adding more lubricating oil.

The amount of oil-in-water remained in the control treatment was increasing everyday resulted from the addition of lubricating oil (Figure 4.8). During the experiment, all forms of chitosan-immobilized bacteria provided the lower amount of remaining oil than in control. This demonstrated that chitosan-immobilized cells had the potential to remove oil even when the concentration of oil-in-water emulsion was increased. Theses results might be described by the increasing of bioavailability of lubricating oil. When oil-in-water emulsion was sorped on chitosan surfaces and its pores, the bacteria cells in chitosan were in closer contact with their carbon source (lubricating oil) and could rapidly degrade it.

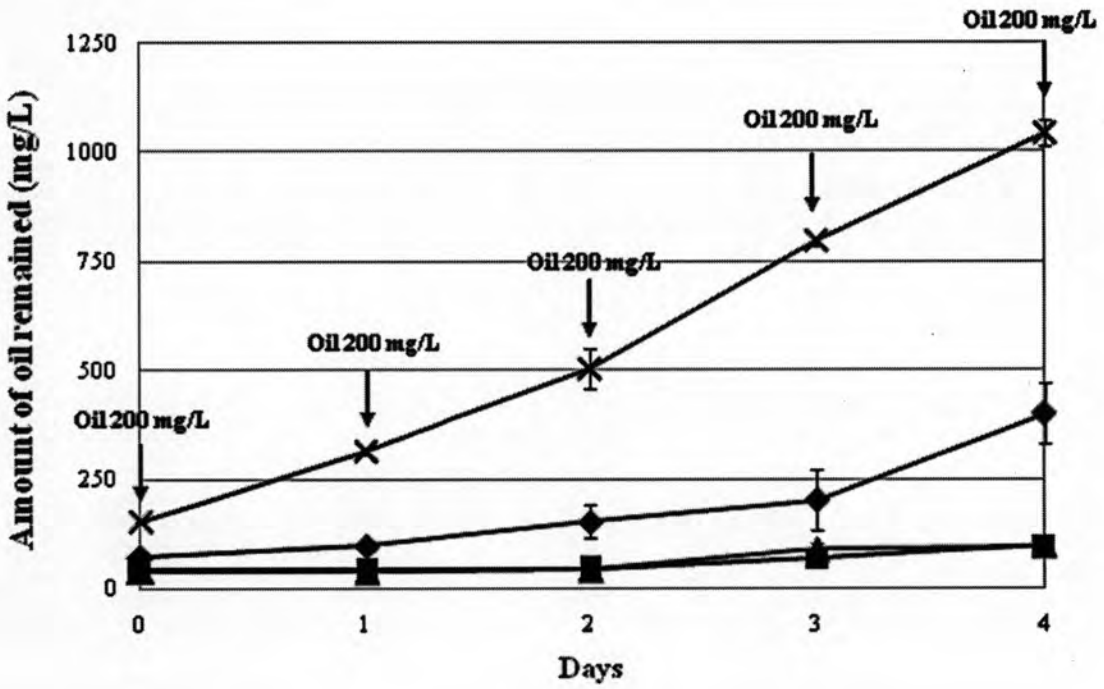


Figure 4.8 Treatment of oil-in-water emulsion by bacteria CH2 immobilized on chitosan: (a) powder chitosan (—◆—); (b) flake squid pen chitosan (—▲—); and (c) flake shrimp shell chitosan (—■—). Control was the oil-in-water emulsion without immobilized cells (—×—).

The results from the treatment of oil-in-water emulsion by three forms of chitosan-immobilized cells were compared between each other. It was observed that the microcosms inoculated with bacteria immobilized on flake shrimp shell and flake squid pen chitosan achieved the highest oil-in-water emulsion removal efficiency and the results were constant throughout the study. In contrast, bacteria immobilized on powder chitosan achieved the lowest oil-in-water emulsion removal efficiency and the amount of oil-in-water emulsion remaining was increased continuously until the end of experiment. These can be concluded that flake chitosan was the suitable media for the immobilization which provided highest oil-in-water emulsion removal efficiency. The greater oil-in-water emulsion removal of the bacteria immobilized on flake chitosan form might be explained by the higher number of bacteria cells survived in flake chitosan during the experiment and the outgrowth bacteria cells from flake surfaces and pores into emulsion. This explanation was later confirmed by the determination of the number of bacteria survived in chitosan and emulsion after treatment.

4.4.2 Number of bacteria survival in chitosan and emulsion

The populations of bacteria survived in chitosan and emulsion after the treatment of oil-in-water emulsion were determined by plate count technique. Figure 4.9 show the survival of bacteria immobilized on three forms of chitosan during the experiment. The initial number of bacteria immobilized in powder chitosan, flake shrimp shell chitosan, and flake squid pen chitosan were 2.33×10^{9}, 2.33×10^{8}, and 1.67×10^{8} CFU/g chitosan respectively. There was no significant change of the number of bacteria in flake shrimp shell during the treatment of oil-in-water emulsion in which the bacteria number was ranging from 2.33×10^{8} to 6.67×10^{8} CFU/g chitosan. However, there was significant change in the number of bacteria in flake squid pen chitosan and powder chitosan during the treatment of oil-in-water emulsion. The number of bacteria survived in flake squid pen chitosan after the treatment was increased approximately 10 times, in which the number was ranging from 1.67×10^{8} to 1.67×10^{9} CFU/ g chitosan. In contrast, the number of bacteria survived in powder chitosan after the treatment was decreased approximately 10 times, in which the number was ranging from 2.33×10^{9} to 8.67×10^{7} CFU/g chitosan. The decreasing of

bacteria survived in powder chitosan after the treatment of oil-in-water emulsion corresponded with the results from 4.4.1 which found the decreasing of oil-in-water emulsion removal efficiency after day 1. Moreover, the decreasing of bacteria survival in powder chitosan during the experiment might be resulted from the effect of the high amount of oil sorped into powder chitosan, which later could be toxic to the immobilized bacteria.

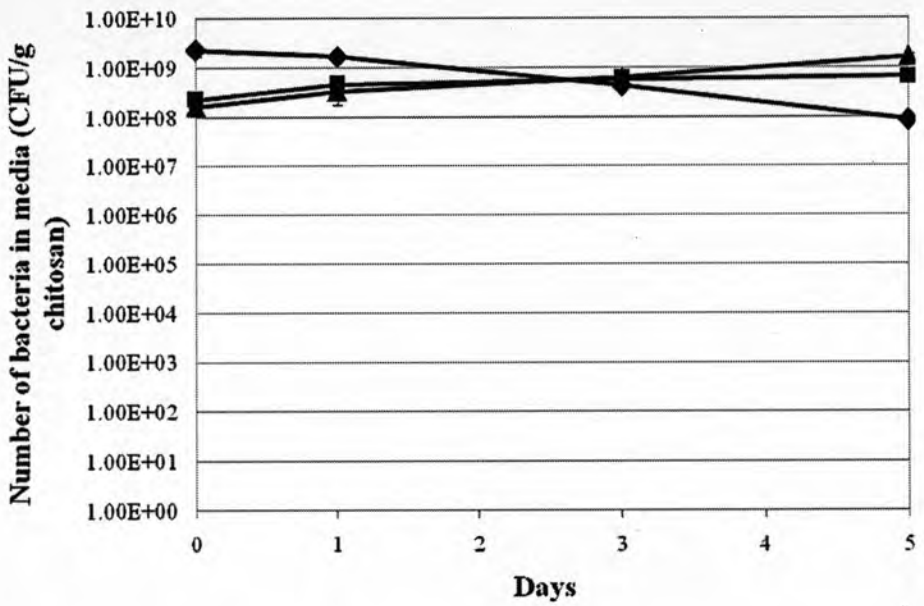


Figure 4.9 Number of bacteria survival in chitosan during the experiment of oil-in-water emulsion treatment by bacteria immobilized on three forms of chitosan: (a) powder chitosan (—◆—); (b) flake squid pen chitosan (—▲—); and (c) flake shrimp shell chitosan (—■—).

Figure 4.10 show the number of bacteria survived in emulsion during the treatment of oil-in-water emulsion by three forms of chitosan-immobilized cells. From the experiment, there were no bacteria observed in the emulsion before the addition of chitosan-immobilized cells into the emulsion. When the treatment of oil-in-water emulsion by chitosan-immobilized cells was proceeded, there were bacteria populations in the emulsion phase. This demonstrated that some bacteria were detached from chitosan after the chitosan-immobilized cells were used to treat oil-in-water emulsion. According to Figure 4.10, there was significant change in the number of bacteria in emulsion after treated by flake chitosan-immobilized cells. The number

of bacteria in emulsion after treated by flake squid pen and flake shrimp shell chitosan-immobilized cells was increased from 2.00×10^5 to 1.33×10^7 CFU/ g chitosan and 6.33×10^5 to 6.30×10^6 CFU/g chitosan respectively. However, there was no significant change of the number of bacteria in emulsion after treated by powder chitosan-immobilized cells in which the number was ranging from 3.00×10^5 to 3.67×10^5 CFU/g chitosan. These results might be described by the different surface characteristics of each chitosan. Powder chitosan exhibited the smooth surface layer; whereas, flake chitosan exhibited the patchy and bumpy surface layer (Figure 4.11). Thus, flake chitosan was probably suitable for the bacterial attachment and the overgrown populations were later detached into the emulsion. In addition, these results can be used to explain the results in 4.4.1. The increasing of bacteria populations in emulsion after treated by flake squid pen and flake shrimp shell chitosan-immobilized cells was probably led to the degradation of the free oil and thereby caused the high oil-in-water emulsion removal efficiency in this study.

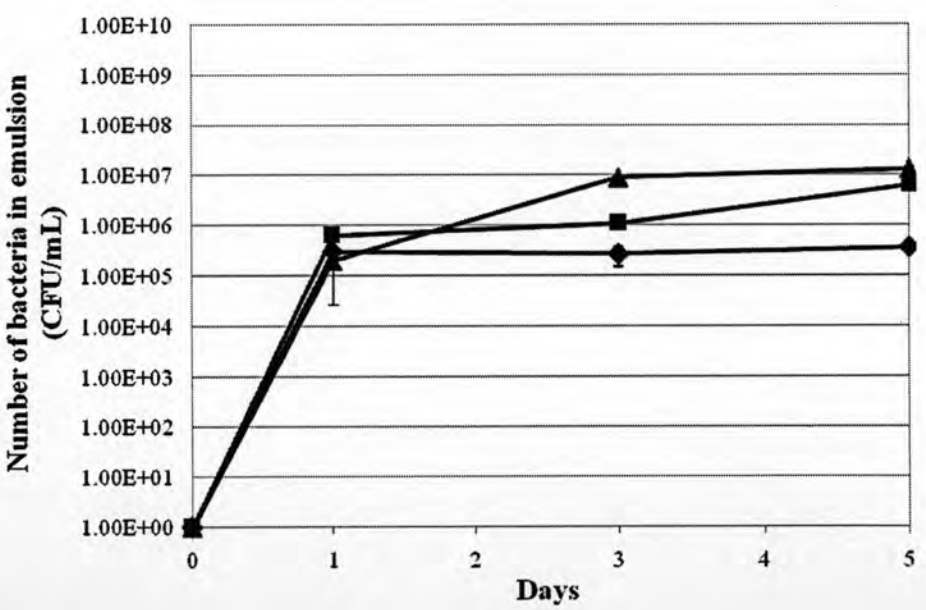
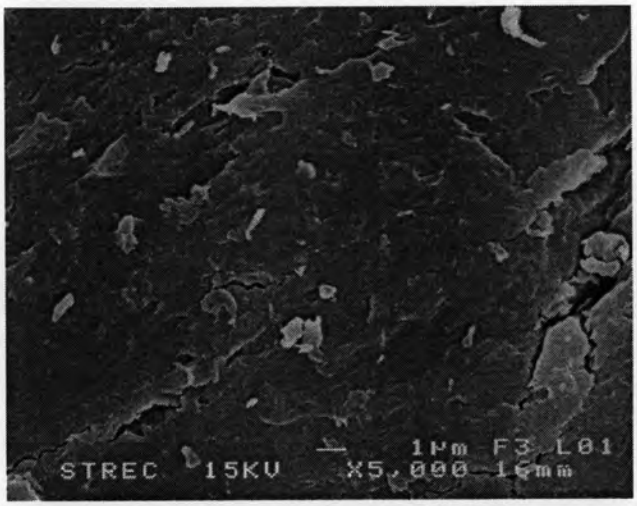


Figure 4.10 Number of bacteria in emulsion during the experiment of oil-in-water emulsion treatment by bacteria immobilized on three forms of chitosan: (a) powder chitosan (—◆—); (b) flake squid pen chitosan (—▲—); and (c) flake shrimp shell chitosan (—■—).

4.4.3 Scanning electron microscope (SEM) of chitosan-immobilized cells

Three forms of chitosan before and after immobilized were analyzed by scanning electron microscope (SEM). The immobilized chitosan was washed with carbon free mineral medium (CFMM) and filtrated through sterilized filter paper for removing unattached cells. Then, a filtrated immobilized chitosan was air dried in sterile hood for 4 hours. Finally, all of dried chitosan samples were coated with gold (Au) before SEM analysis. Figure 4.11 presents the SEM photograph of powder and flake chitosan before immobilize the bacteria. Powder chitosan, flake shrimp shell chitosan, and flake squid pen chitosan, had a different surface layer. The chitosan powder showed a clear and smooth surface layer. Whereas, flake shrimp shell and flake squid pen chitosan have patchy and bumpy surface layer. The different surface layer of these three chitosan probably affected the attachment of bacteria populations and also the treatment of oil-in-water emulsion by these chitosan-immobilized cells.

Figure 4.12 presents a significant change of the layer structure and appearance on powder and flake chitosan after used to immobilize Ch2 bacteria. The SEM photographs showed the consortium of Ch2 bacteria population attached on chitosan surface. Moreover, bacteria populations were aggregated on the fracture of flake chitosan; whereas, scattering bacteria populations were found on the surface of powder chitosan. These results corresponded with Gentili *et al.* (2006), in which *R. corynebacteriorides* strain QBTo was aggregated on chitin and chitosan after immobilization. These chitin and chitosan-immobilized cells were used in the treatment of crude oil contaminated in sea water.



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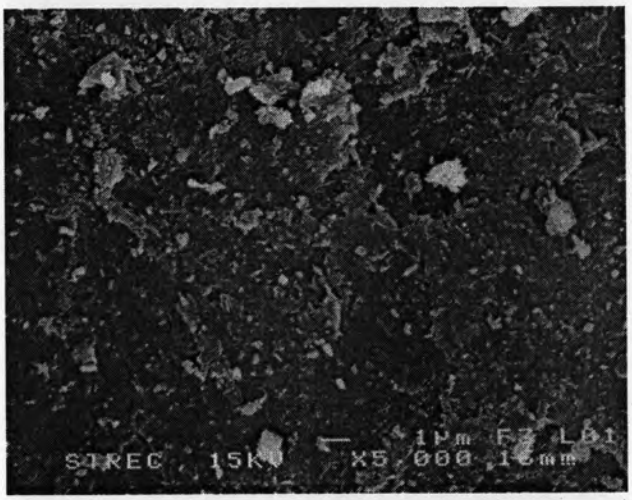

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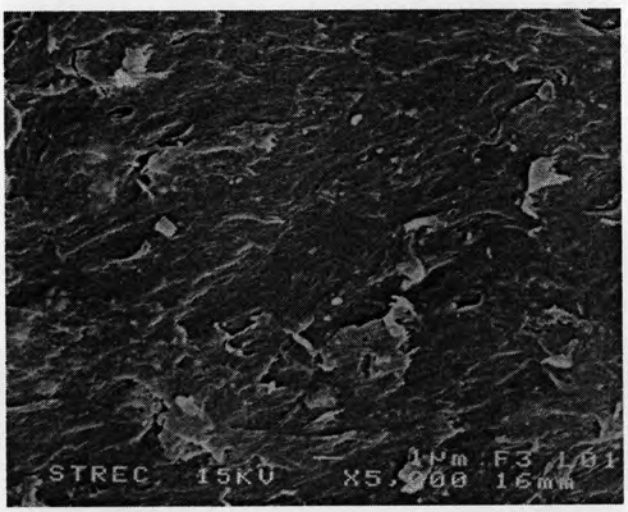
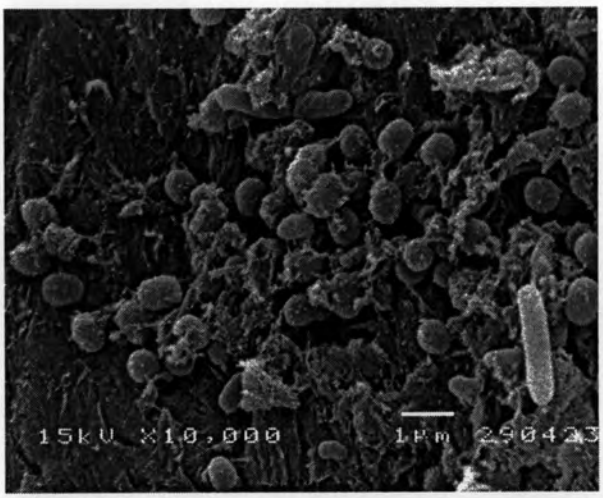


(c)

Figure 4.11 SEM photographs (a) powder chitosan, (b) flake shrimp shell chitosan, and (c) flake squid pen chitosan before immobilize bacteria Ch2. Magnification 5,000x



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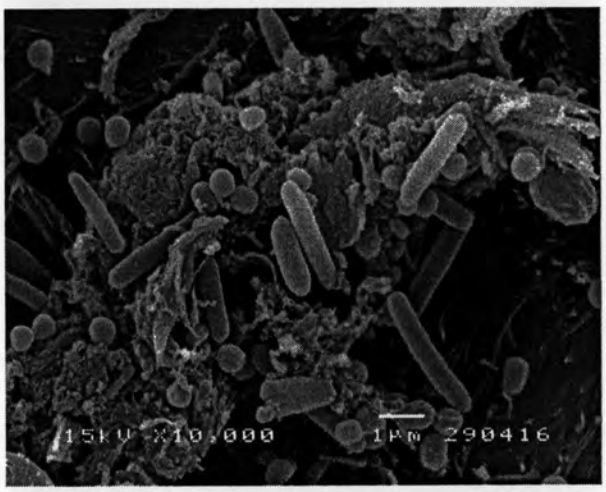

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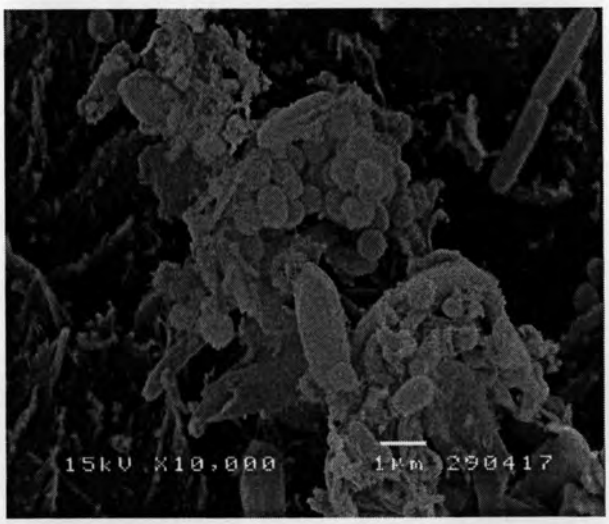

(c)

Figure 4.12 SEM photographs of (a) powder chitosan, (b) flake shrimp shell chitosan, and (c) flake squid pen chiton after used to immobilize bacteria Ch2. Magnification 10,000x.

4.5 Effectiveness of sorption and degradation approach by chitosan-immobilized cells

This experiment compared three types of treatment including sorption by chitosan, degradation by isolated bacteria, and sorption and degradation by chitosan-immobilized cells. Powder chitosan was selected in this experiment since powder chitosan exhibited the highest oil sorption capacity. Flake squid pen was selected to use in the sorption and degradation by chitosan-immobilized cells since flake chitosan-immobiliozed cells achieved higher oil-in-water emulsion removal efficiency than powder chitosan-immobilized cells. The experiments were performed as in section 4.4. There was found that the amount of oil-in-water remaining in water was increased when the lubricating oil was added to the emulsion everyday (Figure 4.13). There was free oil formed on the free water surface after lubricating oil was added.

For the sorption by chitosan treatment, powder chitosan was applied in this experiment using it optimum condition from section 4.2. Powder chitosan achieved the highest oil-in-water emulsion removal efficiency in the first 2 days (Figure 4.13). However, when the concentration of oil-in-water emulsion was increased from 600 to 1000 mg/L, the oil-in-water emulsion removal efficiency of powder chitosan was rapidly decreased. These results can be explained by the maximum oil sorption capacity of powder chitosan which obtained from water and oil sorption by chitosan study. Due to the maximum oil sorption capacity of powder chitosan was 0.48 g oil/g chitosan, chitosan 1.0 g/L can sorp maximum oil concentration at 480 mg/L (Table 4.1). When the concentration of oil remaining in water was higher than 480 mg/L, powder chitosan have no capability to sorp more oil. Hence, powder chitosan achieved low oil-in-water removal efficiency at high oil-in-water emulsion concentration.

For the degradation by isolated bacteria, bacteria Ch2 strain was applied in this experiment. As the results shown in Figure 4.13, bacteria Ch2 achieved the high oil-in-water emulsion removal efficiency in the first 2 days; however, the oil-in-water emulsion removal efficiency of bacteria Ch2 was rapidly decreased when the concentration of oil-in-water emulsion was increased from 600 to 1000 mg/L. This was probably due to the high amount of lubricating oil, which might be toxic to the lubricating oil-degrading bacteria (Environmental Protection Agency, 1995). Thus,

some of bacteria population in the oil-in-water emulsion could not survive when the concentration of oil-in-water emulsion was increased. This explanation can be confirmed by Figure 4.14 which the number of lubricating oil-degrading bacteria was significantly decreased 100 times (from 1.10×10^{7} to 2.03×10^{5} CFU) when the concentration of oil-in-water emulsion was increased during the treatment.

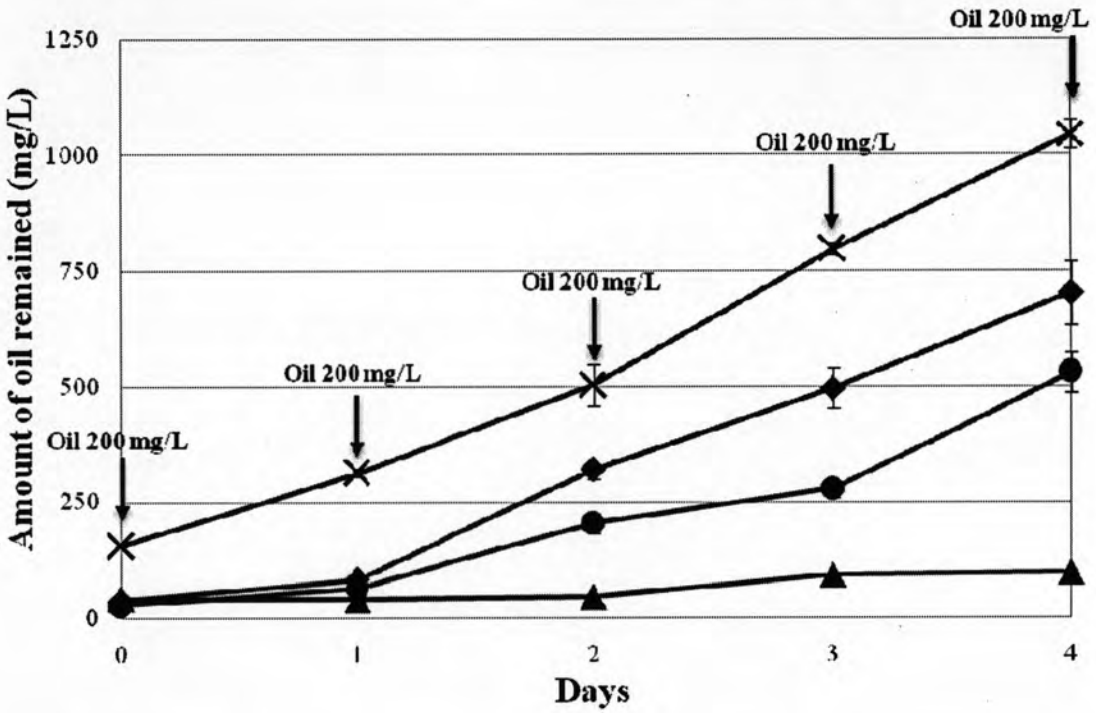

Figure 4.13 Amount of oil-in-water emulsion remaining after treated by three forms of treatment: (a) sorption by chitosan (—◆—), (b) degradation by the isolated bacteria (—●—), and (c) sorption and degradation by chitosan-immobilized cells (—▲—). Control was the oil-in-water emulsion without any treatment (—×—).

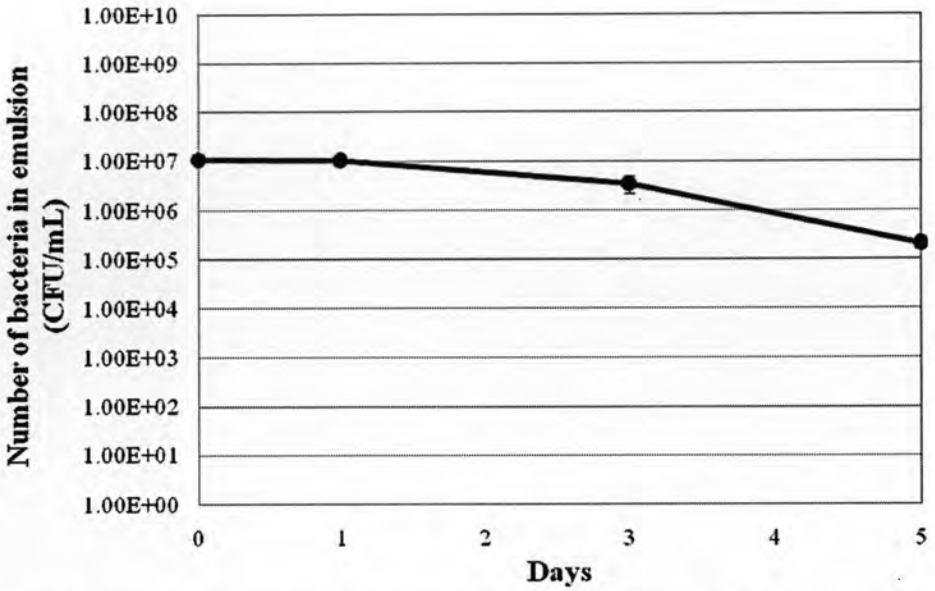

Figure 4.14 Number of bacteria survival in emulsion during the experiment of oil-in-water emulsion treatment by lubricating oil-degrading bacteria

For the sorption and degradation by chitosan-immobilized cells treatment, flake squid pen chitosan was applied in this experiment. When the concentration of oil was increased, the oil-in-water emulsion removal efficiency of chitosan-immobilized cells was still high and relatively constant throughout the study (Figure 4.13). These results suggested that the oil was sorped and simultaneously degraded by chitosan-immobilized cells. Moreover, the treatment could solve the problem of high concentrations of oil in the oily wastewater.

To confirm that the oil sorped in chitosan was degraded by the bacteria immobilized on chitosan, the amount of oil remaining in flake squid pen chitosan-immobilized bacteria and powder chitosan were analyzed. The amount of oil accumulated in powder chitosan was much higher than the amount of oil in chitosan-immobilized cells at all time points (Figure 4.15). The amount of oil in chitosan-immobilized cells was closed to zero; whereas, the amount of oil in powder chitosan was very high and increased continuously. These results suggested that the sorped-oil was rapidly degraded by the immobilized bacteria, thereby preventing the oil accumulation on chitosan surface.

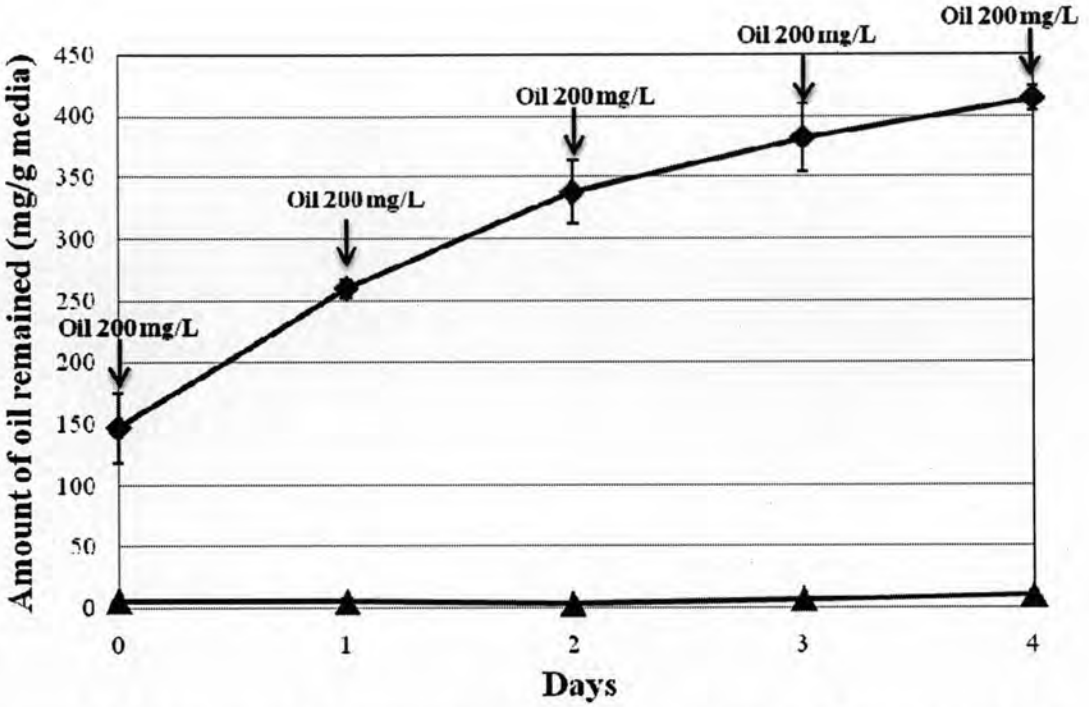

Figure 4.15 Amount of oil remained in (a) chitosan-immobilized bacteria (—▲—) and (b) powder chitosan (—◆—).

As the main components of lubricating oil are saturated and aromatic compounds. Percent removal of saturate and aromatic fraction in lubricating oil was later calculated by comparing with control at day 4 (Figure 4.16). There was found that the sorption and degradation by chitosan-immobilized cells technique achieved the highest percent of saturates and aromatics removal and followed by the degradation by the isolated bacteria and sorption by chitosan technique, respectively. The higher percent aromatics removal than percent saturates removal was found from the oil-in-water emulsion treatment by isolated bacteria and chitosan-immobilized cells. Percentages of aromatics removal in the treatment by isolated bacteria and chitosan-immobilized cells were 55 and 94, respectively. Whereas, percent saturates removal in the treatment by isolated bacteria and chitosan-immobilized cells were only 45 and 89 respectively. This higher aromatics removal was corresponded to the activity of bacteria Ch2, which had the potential to degrade aromatic fraction more than saturate fraction. However, there was not much different between percent aromatics and saturates removals in the treatment of oil-in-water emulsion by powder chitosan which were 34% and 32% respectively. These results indicated that the

removal of oil-in-water emulsion by powder chitosan was only occurred by sorption and coagulation mechanism.

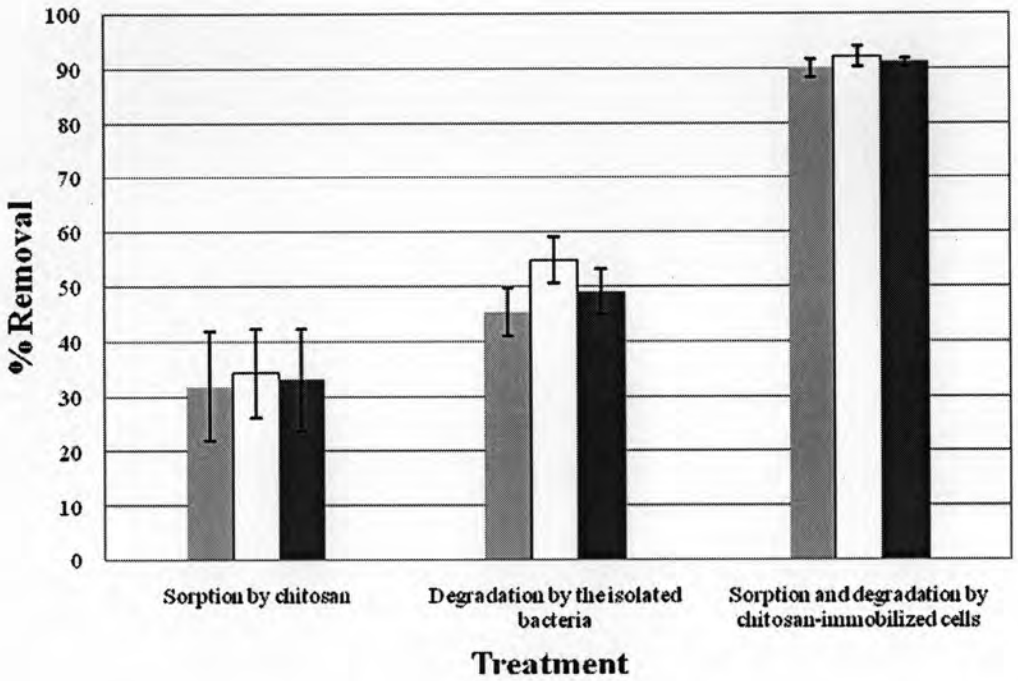

Figure 4.16 Percent removal of saturate (■), aromatic (□) and total oil (■) fractions comparing to control after treatment: (a) sorption by chitosan, (b) degradation by the isolated bacteria, and (c) sorption and degradation by chitosan-immobilized cells. Control was the oil-in-water emulsion without any treatment.

It is well known that bacteria attached on chitosan surfaces and its pores were protected from environmental stress thus the attached bacteria can tolerate and degrade the high amount of oil. According to Gentili *et al.,* (2006), they also showed the high potential of bacteria immobilized on chitin and chitosan flakes to remove crude oil contaminated in seawater. They found that the highest crude oil biodegradation rates were obtained in the microcosms inoculated with *R.corynebacteriorides* QBTo immobilized on to chitin and chitosan flakes. Moreover, the microcosm inoculated with QBTo-immobilized cells provided the higher crude oil removal than microcosm inoculated with the QBTo alone. Oh *et al.* (2000) studied the use of oil-degrading yeast-immobilized polyurethane foams to absorb and degrade crude oil from water surface. They found no significant different between oil-

degradation by free yeast cells and immobilized cells. These results of Oh *et al.* (2000) different from this research which the chitosan-immobilized cells achieved the higher oil removal than free bacteria cells.

The treatment of oil-in-water emulsion from gas station had been carried out by other techniques. Panpanit *et al.* (2002) investigated the potential of Ultrafiltration (UF), Nanofiltration (NF) membrane and advanced oxidation processes (AOPs) for treating oil-in-water emulsion from car wash wastewater. They found that the percent TOC removal by nanofiltration is significantly higher than the ultrafiltration. The average TOC removal was about 60-75% for ultrafiltration and 99% for nanofiltration. For the advanced oxidation process (AOPs), the ozone and peroxone (mixture of ozone and hydrogen peroxide) are not effective for oil/water emulsion removal. At an ozone concentration of 33 mg/L and contact time 60 min, the maximum oil removal in terms of TOC is about 30%. Moreover, Sahairaksa (1999) reported the percent oil removal in the treatment of gas station wastewater by GAC (granular activated carbon), resin, and straw were 87, 79, and 80, respectively.

Percent oil removal of chitosan-immobilized bacteria was comparable to oil sorption by GAC in Sahairaksa (1999). However, oil-sorped GAC will require further treatment as hazardous material. When compared to ultrafiltration (UF), nanofiltration (NF) membrane and advanced oxidation processes (AOPs), the cost of treatment by chitosan-immobilized bacteria is much cheaper. Thus, sorption and degradation by chitosan-immobilized bacteria treatment is recommended for the treatment of oily wastewater from gas station. Furthermore, this treatment is environmental friendly; whereas the oil will be completely degraded and chitosan-immobilized bacteria can be reused.